

Physico-Chemical Characteristics of Date Seed Oil Grown in Sudan

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Abstract: Problem statement: Studies were conducted on the physical-chemical properties of Sudanese date seed oil extracted from two date palm cultivars (*Phoenix dactylifera* L.) *ALBarakawi* and *Alqundeila*. The extracted oil from *ALBarakawi* seeds weighed 6.833% w/w oil, *Alqundeila* oil extracted weighed 5.064% w/w. **Approach:** The physical properties of *ALBarakawi* oil were: viscosity: 17cp, refractive Index: 1.444, density: 0.9116 g cm⁻³ color: Within the range 1. 6-11.1 (red-yellow). For *Alqundeila* the physical properties were: viscosity: 20cp, refractive index: 1.458, density: 0.9174 g cm⁻³ color: 1.8-12.1 (red yellow). **Results:** The chemical properties were: Acid value 2.55, saponification value 0.255 mg g⁻¹, iodine value 71.12 gm I₂/100 gm fat, peroxide value: 4.8 mg. Peroxide/Kgoil for *ALBarakawi*. For *Alqundeila* they were: acid value 2.47, saponification value 0.267 mg g⁻¹ and iodine value 83.31gm I₂/100 gm fat, peroxide value: 7.4 mg Peroxide/Kgoil. In this study, High Performance Liquid Chromatography (HPLC) was utilized for the identification and quantification of vitamin E in these samples. The content of vitamin E for *ALBarakawi* was 5.821 and 6.054 ppm for *Alqundeila*. For the fatty acid content in these oils, Gas Chromatography-Mass Spectrometry (GC-MS) was used. The constituents of fatty acids (calculated % relative to total fatty acid constituents) in *ALBarakawi* were: Saturated lauric acid 37.10%, palmitic acid: 9.24%, Stearic acid 1.71%, Unsaturated fatty acids: Linoleic acid (Omega-6) 4.33%, Oleic acid (Omega-9) 32.66%. For *Alqundeila*, the found fatty acids were: Saturated lauric acid 0.11%, Palmitic acid: 0.42%, Stearic acid 46.93%. **Conclusion/Recommendations:** The elements types and contents in these oils were: Iron 0.27 ppm, Magnesium 0.204 ppm, Sodium 60 ppm, Potassium 470 ppm, Calcium 25 ppm, Selenium 34.4 ppb for *ALBarakawi*. The contents of these elements in *Alqundeila* were: iron 0.27 ppm Magnesium 0.07 ppm, Potassium 2.3 ppm, Selenium 12.6 ppb. The methods involved in this study covered, Gas Chromatography-Mass Spectrometry (GC-MS), High Performance Liquid Chromatography (HPLC), Atomic Absorption Spectroscopy (AAS) and Refractometry. Physico-chemical properties of date seed oil reported in this study were found similar to most of the early published results in this field.

Key words: Gas Chromatography-Mass Spectrometry (GC-MS), High Performance Liquid Chromatography (HPLC), non-natural source, date seeds oil

INTRODUCTION

Return to the natural sources of various food constituents (vitamins, minerals, fats, proteins...). Is becoming the target for researchers throughout the world. This return is caused by the growing recognition of hazards to the human health by foods of non-natural source. The palm tree date forms one of the sources of food for humans and animals in addition to its

economic benefits. Most of the Mediterranean and Arabian Peninsula countries are considered date producing countries. Sudan is also ranked among these countries. Different types of local palm trees are grown in Sudan. They are named *ALBarakawi-Alqundeila-ALMishriq wad Khatib-wad Laqai-ALTamudhah AL Kullimah*. The fruit of the date is composed of a fleshy peri-carp and seed. The date seeds as in most countries was finding little use as no research work was done on

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its possible useful constituents. However recent work including work done by (Besbes *et al.*, 2005; 2004a; 2004b; Chiew *et al.*, 2007; Devshony *et al.*, 1992) proved that date seeds have extractable high value added components. The potential uses of date seed and its constituents are expected to cover uses in cosmetics, medicine, pharmaceuticals, feed and food industries (Harry, 1936; Salem and Hegazi, 1971; E1-Shurafa *et al.*, 1982; Whaibi *et al.*, 1985; Whaibi and Basalah, 1986; Mossa *et al.*, 1986). The literature survey revealed that no previous work was done on Sudanese palm tree seeds oil to evaluate its physico-chemical characteristics or any other possible potential applications. The aim of this work was to extract date seed oil and study its quality characteristics in two selected palm tree types named *ALBarakawi* and *Alqundeila*. This work was also intended to stimulate further research on date seed oil to cover stability, phenolic compounds contents and to point out a constituent that can be considered a marker for the different type palm of trees in Sudan.

MATERIALS AND METHODS

Materials:

Seeds collection: The seeds of two cultivars: *ALBarakawi* and *Alqundeila* were collected from a local date's market at Omdurman city. (Major source of the collected dates is Northern Sudan).

Extraction methods:

Oil extraction and preparation: Weights of about 750-900g of *ALBarakawi* and *Alqundeila* seeds were used for oil extraction. The seeds were separated from the fruits, dried and crushed into minute granules using automatic machine (retsch-100) for 3 m. Total lipids were extracted from the seeds with hexane using Soxhlet apparatus. The extraction process continued for 4-6 h. The solvent was evaporated on rotary evaporator under reduced pressure and the produced oil (free from n-hexane) was collected, weighed, stored in a dark container in a deep-freeze (-20°C) till subsequent analyses.

Fatty acids composition: The oil fatty acid methyl esters were prepared as described in Christie (AOAC, 1990). One ml of the produced oil was added to seven ml methanolic NaOH (0.5 M) followed by seven ml of methanolic H₂SO₄ before shaking. The solution was left overnight. Two ml of n-hexane or heptane was added to the reaction mixture followed by saturated NaCl. This mixture was shaken well and allowed to separate into two layers. One ml from the upper layer was transferred to a new tube and dried with anhydrous Na₂SO₄.

Analytical methods:

Viscosity determination: Viscosity was determined at 30°C using HAAKE viscometer 6 plus (thermo-electron corporation instrument type 387-0100).

Determination of Refractive index: The Refractive index was determined by using BELLINGHAM and STANLEY L^{TD} LONDON-(No 918095 MADE in ENGLAND, instrument). Refractometer was adjusted first, using distilled water.

Density determination: Density was measured using Puchnometer Kit for AEP balances, (Adam Equipment co.L^{TD}. Bond Avenue Denbigh East Estate Milton Keynes, MK, ISV-united kingdom. (AEP-250g-max 250 g⁻¹), d = 1mg, density determination = g/c³).

Colour determination: The Colour was determined by using Lovibond or Tintometer typed" instrument (Tintometer L^{TD}. The colour laboratory Salisbury England).

Vitamin E identification and determination by

HPLC and GC-mass: Vitamin E in the oil sample (0.2 gm mL⁻¹ solution in n-hexane) was identified and evaluated by HPLC and GC-mass. The quantification of Vitamin E in the oils was determined using standard vitamin E solution containing 2000 ppm diluted to obtain a calibration curve ranging between 20-2000 ppm.

HPLC system: Analyses were carried out using Water/U.S.A600 High Performance Liquid Chromatography (HPLC), pump 600, Detector 2969 Photo Diode Array (PDA) Stationary phase: Normal Phase Silica (NP-HPLC)Column (4.6×150 mm), flow rate 1.0 mL min. Injector volume 20 µL⁻¹, Wavelength 295 nm. Mobile Phase composition:

(n- hexane + THF + 2- propanol) (500 + 30 + 2)

GC-Mass system: Analyses were carried out using a Varian 450 Gas chromatography equipped with Varian 220-IT Mass spectrometer and VF-5 ms capillary column (30×0.25 mm; 0.25 µm film thickness). The oven temperature was held at 50°C for 5 min and programmed at 7°C/min to 300°C for 5 min. Other operating conditions were: Helium gas carrier at a flow rate of 1.0 mL⁻¹ min. injector and detector temperature were 250 and 280°C respectively; split ratio 1:50. Mass spectra were taken at 70 ev mass ranges from m/z 40-400 amu.

GC-MS identification and determination of fatty acids methyl esters in the oils: Gas Chromatography

Mass Spectrometer was performed using Shimadzu instrument (GC-MS-QP-2010) fitted with electron impact (EI 1.70 eV) mode. The analytical column was RTX 5 (5% phenyl-95% dimethyl polysiloxane with length of 30 m × 0.25 μm). Helium gas was used as a carrier gas at a flow rate of 1 mL min⁻¹. The temperature was programmed at 40°C for 0 min then increased to 150°C at the rate of 15°C/min with hold time 5 min then increased to 250°C with hold time 10 min. The temperature of injector was 250°C.

AAS identification and determination of elements in the oils: Atomic Absorption Spectroscopy was performed using Shimadzu instrument (AAS-6800).

For the Mg, Fe, Ca, Na, K, 10 mL oil sample was dissolved in 1 mL carbon tetrachloride (CCl₄). To this solution 10 mL⁻¹ 10% v/v nitric acid was added, mixed well for about 10 min before centrifugation at 2500-3000 revolution per minute for 2 min. The supernatant was then separated and the nominated elements were determined by AAS with reference to their respective standards. For selenium the same above procedure was followed but using nickel sulphate 0.25 gm dissolved in 10 mL of 10% v/v nitric acid (Price, 1979).

RESULTS

Table 1 reflects the physico-chemical characteristics of the extracted seeds oil of the two date palm cultivars (*ALBarakawi* and *Alqundeila*) compared with characteristics of some reported values cited in the Literature (Besbes *et al.*, 2005; Devshony *et al.*, 1992).

The oil was a semi-solid at temperatures below 10°C and a viscous liquid at a ambient temperature.

The fatty acids % content calculated using internal normalization method (in descending order were) lauric acid (37.10%), Oleic acid (32.66%), Methyl tetradecanoate (14.73%), Palmitic acid (9.24%), Linoleic acid (4.33%), Stearic acid (1.71%) and finally 1, 2 Benzenedicarboxylic acid (phthalic acid) (0.22%)..

Figure 1 reflects a typical GC chromatogram of the separated fatty acids and Table 2 reflects their relevant retention times.

Figure 2a shows a peak for α-tocopherols at about 2.5 min retention time for the standard. Figure 2b shows typical chromatogram of sample showing vitamin E at: (RT: 2.535) and a number of unidentified peaks.

Table: 1 Reflects the summarised physico-chemical properties of the two oils compared with previously reported results

Date seeds oils	Cultivars					
	<i>ALBarakawi</i>	<i>Alqundeila</i>	Besbes <i>et al.</i> (2005)	s.Devshony <i>et al.</i> (1992)		
oil extracted	6.833% w/w	5.064% w/w	NR*	NR*	8.40%	8.13%
Oil^{bhc}						
Viscosity	17cp	20cp	18.50mpa.s	20.50mpa.s	NR*	NR*
Refractive Index at(20°C)	1.444	1.458	1.457	1.462	NR*	NR*
Density	0.9116g/cm ³	0.9174 g/cm ³	NR*	NR*	NR*	NR*
Colour	1.6-11.1	1.8- 12.1	NR*	NR*	NR*	NR*
Acid value (mgKOH/goil)	2.55	2.47	NR*	NR*	NR*	NR*
Specification value	0.255mg/g	0.267 mg/g	NR*	NR*	NR*	NR*
Iodine value (Wijs)	71.12 gm I ₂ /100 gm fat	83.31 gm I ₂ /100 gm fat	45.49 g/100g	44.08 g/100g		
Peroxide value	4.8 mg.Peroxide /kg oil	7.4 mg.Peroxide /Kg oil	NR*	NR*	NR*	NR*
Vitamin E ^{HPCLC}	5.821ppm	6.054ppm	24.97±1.40	38.85±1.80	NR*	NR*
Vitamin E ^{GC-MASS}	Structure Vit E	Structure Vit E	NR*	NR*	NR*	NR*
Fatty acid^d						
lauric acid	37.10%	o.11%	24.34%	22.56%	24.00%	19.6%
Palmitic acid	9.24%	o.42%	NR*	NR*	9.6%	10.20%
Stearic acid	1.71%	46.93%			1.30%	1.90%
Oleic acid (Omega-9)	32.66%.	Nd*	39.17%	42.13%	41.10%	44.10%
Linoleic acid (Omega-6)	4.33%	Nd*	NR*	NR*	14.5%	12.00%
Mineral in oils						
Iron	0.27 ppm	0.27ppm	NR*	NR*	1.68%	0.78%
Magnesium	0.204 ppm	0.07ppm	NR*	NR*	7.78%	6.74%
Sodium	60 ppm	Nd*	NR*	NR*	1.48%	1.37%
Potassium	470 ppm	2.3 ppm	NR*	NR*	25.40%	28.90%
Calcium	25 ppm	Nd*	NR*	NR*	1.58%	1.87%
Selenium	34.4ppb	12.6ppb	NR*	NR*	NR*	NR*

Oil^{bhc}: physico-chemical, Nd*: Not detected, NR: Not reported, Fattyacid^d: percent by weight of total fatty acids

Gas Chromatography mass spectrometer QP-2010-SHIMADZU

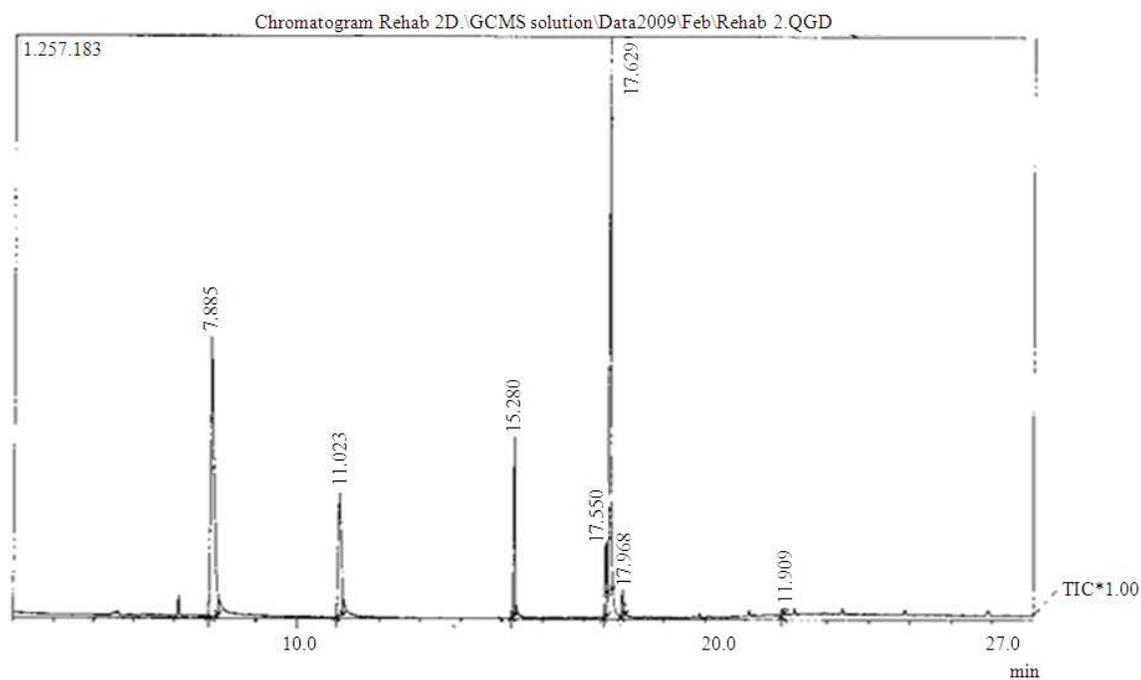
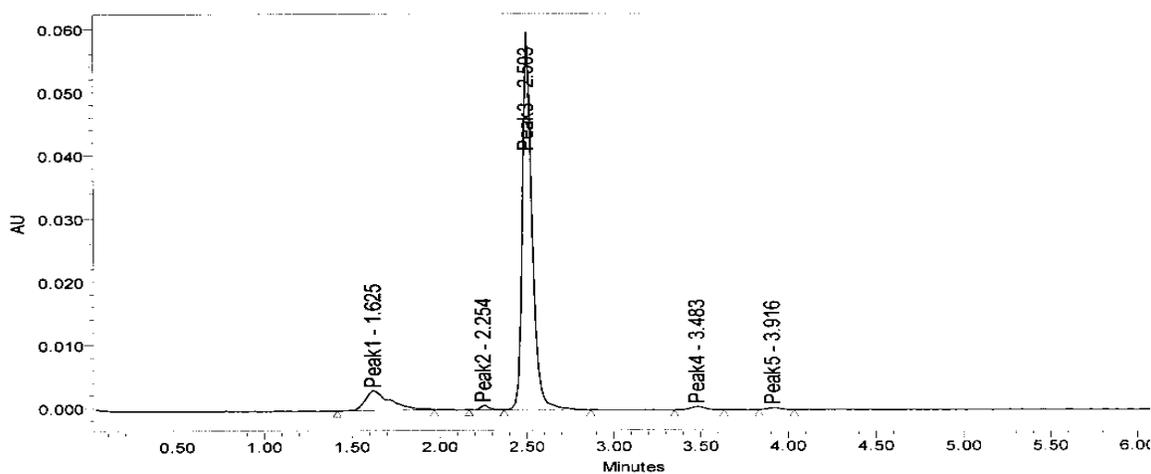
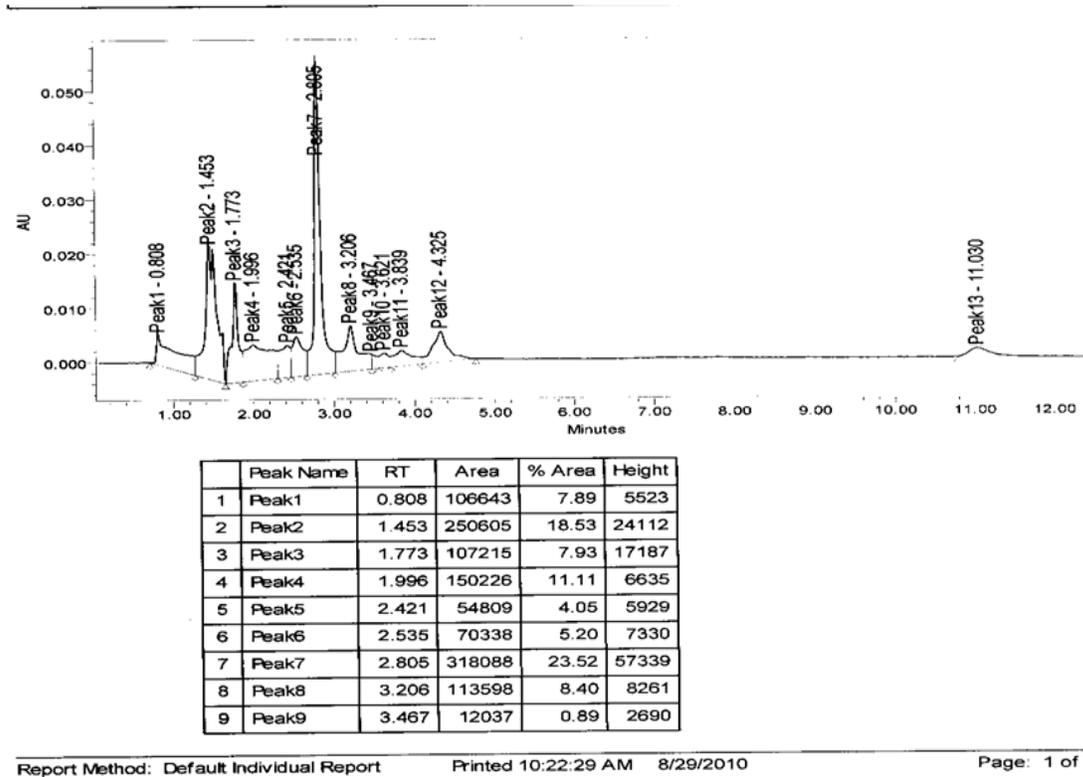


Fig. 1: Typical GC- mass chromatogram of separated fatty acids



	Peak Name	RT	Area	% Area	Height
1	Peak1	1.625	31292	11.53	3120
2	Peak2	2.254	2570	0.95	677
3	Peak3	2.503	232340	85.64	56753
4	Peak4	3.483	3292	1.21	524
5	Peak5	3.916	1812	0.67	306



(b)

Fig. 2a: Typical chromatogram of standard vitamin E (RT: 2.503) (b) Typical chromatogram of sample showing vitamin E at: (RT: 2.535)

Table 2: Reflects the retention times for the GC-mass separated fatty acids

Peak	R. Time	Area %	IUPAC name	Fatty acid
1	7.8850	37.10	Dodecanoic acid	Lauric acid
2	11.0230	14.73	Methyle tetradecanoate	Methyl tetradecanoate
3	15.2800	9.24	Hexadecanoic acid	Palmitic acid
4	17.5500	4.33	9,12-Octadecanoic acid	Linoleic acid
5	17.6290	32.66	9- Octadecanoic acid	Oleic acid
6	17.9680	1.71	Octadecanoic acid	Stearic acid
7	21.9090	0.22	Phthalic acid	1,2 Benzendicarboxylic acid
		100.00		

DISCUSSION

In this study it was found that the chemical constituents which are of interest were the fatty acids constituents and vitamin E content. The seeds oil can be considered a good source of lauric acid and Oleic acid (Omega-9). This is in agreement with the finding reported by (Besbes *et al.*, 2005) for α -tocopheral in their extracted seeds oils Table 1. It is worth noting that the content of Oleic acid and lauric acid in the Sudanese date seed oils were 37.10 and 32.66%

respectively at a ratio of 1.136. On the date seed oils reported by Devshony *et al.* (1992), they were (42.5%) Oleic acid compared to (21%) lauric acid at a ratio2:1. Table 1 also reflects that ALBarakawi summarised physico-chemical properties out-weigh those of Alguendela with the exception of stearic acid contents the other chemical constituent identified in the date seed oils was the naturally occurring antioxidant, vitamin E (tocopherols). Medically, vitamin E is a potent antioxidant that protects the body against oxidation reactions (radicals) that damage membranes

cholesterol transporting lipoproteins. Other medical use cover activity as screening reagent protecting against skin damage and aging by UV radiation, inhibits growth of cancer cells and a protector against atherosclerosis by lowering cholesterol level (Packer, 1991; Traber *et al.*, 1997; Sundram *et al.*, 1989; Goh *et al.*, 1994; Lercker and Rodriguez-Estrada, 2000; Ziegler and Filer, 1996; Rimm *et al.*, 1993). The richest source of vitamins E or tocopherols in the diet is vegetable oils and their products including palm oil (Chiew *et al.*, 2007). Tocopherol in date tocopherols in date seed oil are reported by (Besbes *et al.*, 2005). In our study the date seed oil was also found to contain Vit E (Fig. 2a and b). The semi-solid nature of the oils is an indication of the presence of major saturated and unsaturated fatty acids (lauric acid and oleic acid respectively) The presence of α -tocopherols in the oils is reflected by the coinciding retention times of 2.5 min. for the standard and a corresponding peak in the sample chromatogram (Fig. 2a and 2b). sample chromatogram also shows a number of unidentified peaks which could include (α , β , γ , δ)

tocopherols. The content of α -tocopherols was estimated in this study to be about 6.054 ppm in both studied seed oils compared to a 25-40 ppm reported by (Besbes *et al.*, 2005) for α -tocopherol in their extracted seeds oils Table 1.

Table 3 shows the mass spectral assignments of major Vit E fragments depicted from Fig. 3.

The molecular ion at 430 m/z corresponds to the molecular weight of tocopherol (430). The other fragments presented in the table collectively indicate the tocopherol structure.

Table 3: Shows the mass spectral assignments of major Vit E fragments

M/Z	Species
430	M ⁺
415	(M-CH ₃) ⁺
205	(M-C ₁₆ H ₃₃) ⁺ = (C ₁₃ H ₁₇ O ₂) ⁺
165	(C ₁₀ H ₁₃ O ₂) ⁺ base peak
85	C ₆ H ₁₃ ⁺
71	C ₅ H ₁₁ ⁺
57	C ₄ H ₉ ⁺
43	C ₃ H ₇ ⁺
29	C ₂ H ₅ ⁺

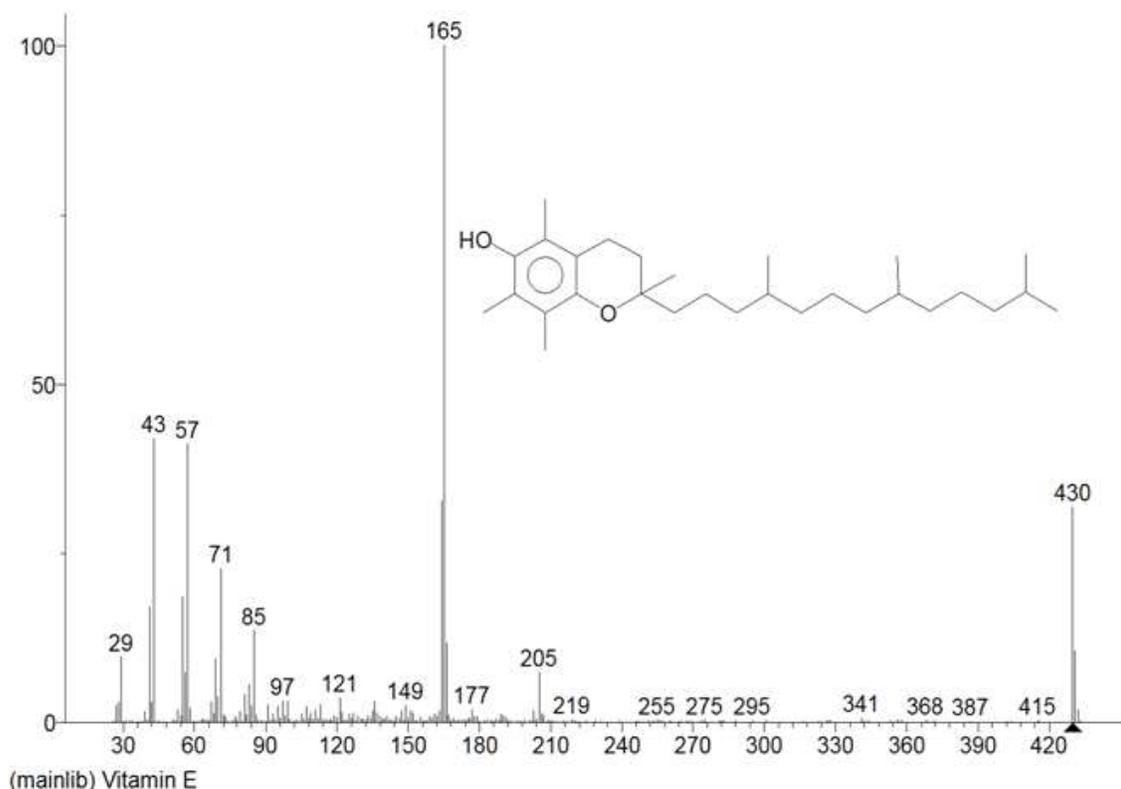


Fig. 3: Vitamin E identification in *ALBarakawi* and *Alqundeila* date seeds oil by GC-MASS

CONCLUSION

The various constituents of date seed oils reported in this study, compared to previous studies (2,6) done in different geographical areas confirmed similarity of the major constituents of date palm trees seeds oil whether grown in tropical or Mediterranean areas.

Joint with these previous studies date seeds oils potential use in industry (food, cosmetics, medicine and pharmaceutical) is promising.

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